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## Effects of dry period energy intake on insulin resistance, metabolic adaptation, and production responses in transition dairy cows on grass silage-based diets

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1 **Interpretive Summary:**

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3 **Effects of dry period energy intake on insulin resistance, metabolic adaptation, and production**  
4 **responses in transition dairy cows on grass silage-based diets**

5 By Salin et al. Periparturient period is associated with insulin resistance in ruminants. Oversupply  
6 of energy during the dry period may have adverse effects on metabolic adaptation, by affecting  
7 maternal insulin resistance, accretion and mobilization of body reserves and feed intake of  
8 periparturient dairy cows. Our study showed that free access to grass silage during the dry period  
9 did not negatively affect metabolic changes or feed intake during the transition period compared  
10 with controlling the energy intake by diluting grass silage with wheat straw.

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13 **ENERGY INTAKE AND INSULIN RESISTANCE IN COWS**

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15 **Effects of dry period energy intake on insulin resistance, metabolic adaptation, and production**  
16 **responses in transition dairy cows on grass silage-based diets**

17

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27 **ABSTRACT**

28 High energy intake in the dry period has reportedly had adverse effects on mobilization of body  
29 reserves, DMI, and productivity of dairy cows. We investigated whether grass silage (GS) fed ad  
30 libitum (HEI; 141% of daily metabolizable energy (ME) requirements) in 8 wk dry period affects  
31 metabolic adaptation and specifically peripheral insulin resistance, compared with ad libitum fed  
32 total mixed ration (TMR) consisting of GS, wheat straw (WS) and rapeseed meal (55/40/5%; 108%  
33 of ME/d; CEI). Multiparous Ayrshire dairy cows (n = 16) were used in a randomized complete block  
34 design until 8 wk after parturition. Commercial concentrates were fed 1 and 2 kg/d during the last  
35 10–6 and 5–0 d before the expected calving date, respectively. Postpartum, a similar lactation diet  
36 with ad libitum access to GS and increasing concentrate allowance (max. 16 kg/d) was offered to  
37 all. HEI group gained more body weight and had higher plasma insulin, glucose, and  $\beta$ -  
38 hydroxybutyrate concentrations than CEI prepartum. Postpartal plasma glucose tended to be higher  
39 and milk yield was greater from wk 5 onwards for HEI than CEI cows. Intravenous glucose tolerance  
40 test (IVGTT) was performed at  $-13 \pm 5$  d and  $9 \pm 1$  d relative to calving. HEI cows had greater  
41 insulin response to glucose load and smaller area under the response curve for glucose than CEI  
42 cows in prepartal IVGTT. Thus, compensatory insulin secretion adapted to changes in insulin  
43 sensitivity of the peripheral tissues, preserving glucose tolerance of HEI. Higher insulin levels were  
44 needed in HEI than CEI cows to elicit a similar decrement of non-esterified fatty acid (NEFA)  
45 concentration in prepartal IVGTT, suggesting reduced inhibition of lipolysis by insulin in HEI  
46 before parturition. In conclusion, high energy intake of moderately digestible GS with low  
47 concentrate feeding in close-up dry period did not have adverse effects on metabolic adaptation,  
48 insulin sensitivity and body mobilization after parturition. Instead, this feeding regime was more  
49 beneficial to early lactation performance than GS-based TMR diluted with wheat straw.

50 **Key words:** insulin resistance, transition dairy cow, energy intake, grass silage

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## INTRODUCTION

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In loose housing systems, group fed dairy cows are prone to extra weight gain after drying-off. Dry cows easily overconsume energy relative to requirements, even when given moderate-energy maize-silage (**MS**)-based diets (Janovick and Drackley, 2010). Although overfeeding may induce only minor visible changes in BCS, overfed cows share common metabolic features with the obese cow (Drackley et al., 2014). These include e.g. accretion of visceral fat depots, which drain directly to the liver predisposing cows to health problems (Roche et al., 2013; Drackley et al., 2014) and decreased DMI in early lactation (Agenäs et al., 2003; Dann et al., 2006; Janovick and Drackley, 2010).

The daily energy intake of dry cows offered feed ad libitum can be controlled by feeding of bulky forages rich in fibre, such as wheat straw (**WS**). Restricting the energy allowance by diluting MS-based diets with WS during the dry period improved DMI and energy balance (**EB**), and decreased hepatic lipid content, and adipose tissue (**AT**) mobilization in very early lactation (Janovick and Drackley 2010; Janovick et al., 2011; Mann et al., 2015). This suggests that restriction of energy intake in the dry period improves metabolic balance in early lactation, despite higher non-esterified fatty acid (**NEFA**) levels in transition period (Douglas et al., 2006; Loores et al., 2006; Roche et al., 2015). However, the results have been more equivocal in dry cows on grass silage (**GS**) based feeding strategies. No beneficial effects on DMI and lipid mobilization were reported when dilution of GS or mixture of grass and MS with straw was compared with pure GS (Ryan et al., 2003, Butler et al., 2011), or when the allowance of GS was restricted to meet the energy requirements (Kokkonen et al., 2018).

75 Some recent studies have shown that prepartal energy intake with or without BCS changes may affect  
76 maternal insulin resistance (**IR**) during the periparturient period (Zachut et al., 2013; De Koster et  
77 al., 2015; Salin et al., 2017). The hyperbolic relationship between insulin secretion and insulin  
78 sensitivity suggests that any environmental change in insulin sensitivity, for instance in response to  
79 obesity, will be compensated by an increase in insulin secretion in response to glucose (Bergman,  
80 1989; Kahn et al., 1993). This hyperbolic relationship in insulin dynamics, well established in human  
81 studies, was also recently verified in transition dairy cows (Salin et al., 2017). As a result of prepartal  
82 overfeeding, the AT may be more refractory to the actions of insulin (Salin et al., 2017), and cows  
83 prone to lose great amounts of BW in transition period may be more resistant to insulin's effect on  
84 lipolysis inhibition than cows with moderate BW loss (Zachut et al., 2013). However, some more  
85 recent studies found minor effects of increased body fatness and high energy intake on inhibition of  
86 lipolysis by insulin, and insulin signaling in AT of transition dairy cows (De Koster et al., 2016a;  
87 Mann et al., 2016, Jaakson et al., 2018). Instead, over-conditioned cows were more insulin resistant  
88 in regard to glucose metabolism and glucose transport into AT in late pregnancy than leaner cows  
89 (De Koster et al., 2015; Jaakson et al., 2018).

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91 In our former experiment (Salin et al., 2017; Kokkonen et al., 2018), we showed that high energy  
92 intake of GS induced a more refractory NEFA response during the prepartal IVGTT when compared  
93 with cows fed with controlled amount of GS to meet ME requirements, whereas a more pronounced  
94 NEFA response was observed in overfed cows after parturition. However, a relatively short dry  
95 period of 6 wk, and a feeding practice where energy oversupply was gradually restricted during the  
96 close-up dry period might have contributed to the absence of differences in accretion and  
97 mobilization of body reserves between high and controlled energy diets. This may have dampened  
98 the metabolic and hormonal effects in the former study. Accordingly, we did not observe effects on  
99 glucose and insulin responses during the transition period.

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101 The main objective of this experiment was to determine whether increased dietary energy level by  
102 ad libitum allowance of GS during an 8-wk dry period, and subsequent putative increase of body  
103 condition during 8 wk dry period deteriorates pregnancy induced IR followed by abnormal metabolic  
104 regulation during the transition period. Our hypothesis was that high dietary energy intake  
105 accelerates accretion and mobilization of body reserves, affects hormonal control of metabolism,  
106 decelerates the increase of DMI in early lactation, and thus affects production responses during the  
107 first 8 wk of lactation. We also assumed that limiting of DMI by increasing the NDF-content of GS-  
108 based diet with adding of WS to TMR would prevent aforementioned negative effects.

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## MATERIALS AND METHODS

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### *Cows, Diets, and Experimental Design*

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The experimental procedures were conducted under the protocols approved by the National Animal Ethics Committee in Finland (Hämeenlinna). The study was conducted with 16 Finnish multiparous Ayrshire cows, using a randomized complete block design. Experimental animals were dried off either before ( $n = 12$ ; average 12.6 d; median 6.0 d) or on the day ( $n = 4$ ) of the initiation of the experimental period. Those that were dried-off in advance were fed refusals of GS until 8 wk prior to expected parturition. The cows were paired according to expected calving date, parity (second through sixth), BW ( $733 \pm 115.1$  kg; mean  $\pm$  SD), and BCS ( $3.5 \pm 0.54$ ; mean  $\pm$  SD). The 305-d milk yield from the previous lactation of the cows was  $10,280 \pm 1,469$  kg (mean  $\pm$  SD). Within pairs, cows were allocated to 1 of the 2 prepartal dietary treatments 8 wk before the expected parturition ( $57 \pm 5$  d before the actual calving day; mean  $\pm$  SD). The dietary treatments were (1) ad libitum access to total mixed ration (TMR) containing a mixture of 1st cut wilted GS (55%, digestible OM = 667 g/kg of DM), WS (40%, digestible OM = 457 g/kg of DM), and rapeseed meal (5%, digestible

124 OM = 700 g/kg of DM) contributing to an average of 587 g/kg of digestible OM and a ME content  
125 of 9.1 MJ ME/kg of DM (controlled energy intake, **CEI**) and (2) ad libitum access to 2nd cut wilted  
126 GS (high energy intake, **HEI**), containing 638 g/kg of DM of digestible OM and a ME content of  
127 10.1 MJ. The silage was ensiled in round bales. Prior to baling a formic acid-based additive (AIV2  
128 Plus, Kemira Ltd, Helsinki, Finland) was applied at a rate 7.4 L/tonne. The preparation of TMR is  
129 described in detail in Selim et al. (2015). Grass silage or TMR was offered to cows 3 times daily at  
130 0700, 1300, and 2000 h. The composition of feeds is presented in Table 1.

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132 Both diets were supplemented with 1 kg/d of commercial concentrate (Pro-Maituri 20, Raisioagro  
133 Ltd., Raisio, Finland) during d 10 to d 6 prior to the expected calving date and with 2 kg/d thereafter  
134 until the day of calving. The chemical composition of the concentrates is shown in Table 1. The  
135 concentrate ration was fed twice daily (at 0615, and 1700 h). A commercial mineral and vitamin  
136 supplement (Tunnu-Melli, Raisioagro Ltd., Raisio, Finland) was top-dressed (0.2 kg/d) once daily  
137 on forage during the dry period (Supplemental Table S1). After calving, all cows were offered wilted  
138 GS (average D-value 687 g/kg DM) ad libitum. The silage was cut and mixed in a stationary mixer,  
139 distributed 4 times per day (at 0500, 1100, 1500, and 2000 h) by a rail-suspended distribution wagon  
140 (Pellon Group, Ylihärmä, Finland). The cows were fed an increasing amount of the same commercial  
141 concentrate as before calving, and a protein and mineral plus vitamin supplement (Amino-Maituri  
142 30; Pihatto-Melli, Raisioagro Ltd., Raisio, Finland; Supplemental table S1). The rate of increase in  
143 daily concentrate ration was similar for all cows (Supplemental table S2). The volume of concentrate  
144 was 8 kg/d by the end of lactation week 1 (7 kg/d of cereal concentrate + 1 kg/d of protein  
145 supplement), the maximum amount of 16 kg/d (12.5 kg/d of cereal concentrate + 3.5 kg/d of protein  
146 supplement) was achieved by lactation d 32. During the first week of lactation, the daily concentrate  
147 ration was fed 4 times per day (at 0615, 1000, 1645 and 1930 h) to separate feeding troughs.

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149 The experimental animals were housed in tie stalls with saw dust bedding and rubber mats  
150 throughout the dry period. The cows had continuous access to automatic water troughs and salt licks.  
151 Approximately 1 wk before the expected calving, the cows were moved to individual calving pens.  
152 The cows were returned to the tie stalls 2 to 5 d after parturition. The tie stalls and the calving pens  
153 were equipped with forage intake control feeding stations (Insentec BV, Marknesse, the  
154 Netherlands), which were fitted with separate concentrate troughs. The cows were kept in tie stalls  
155 until d 10 of lactation and moved to a free-stall barn equipped with Roughage Intake Control (RIC)  
156 system (Insentec BV, Marknesse, the Netherlands) with separate automatic concentrate feeders and  
157 (Lely Cosmix, Lely Industries N.V., Maassluis, the Netherlands).

158

#### 159 ***Feed and Milk Samples, Chemical Analysis, and Measurements***

160 Feed offered, and feed refused (silage and concentrates) were recorded daily. The feeds were  
161 sampled weekly, and the cereal concentrate and silage samples were pooled to form a monthly  
162 sample. Samples of concentrates were pooled to form a 2-mo sample. Feed samples were analyzed  
163 as described by Salin et al. (2012), and dry matter content of silages was corrected for the loss of  
164 volatiles according to Huida et al. (1986). VFA of the silage was determined by liquid  
165 chromatographic analysis using Waters Acquity UPLC chromatography apparatus (Waters, Milford,  
166 MA, USA), as described in detail by Puhakka et al. (2016). Total fat of concentrates was analyzed  
167 with ether extraction and hydrolysis with HCl (SoxCap 2047 Hydrolysis Unit; FOSS Soxtec 8000,  
168 FOSS Analytical, Hilleroed, Denmark). During the first week of lactation, the cows were milked  
169 twice daily at 0630 and 1700 h, and thereafter until lactation week 8, the cows were milked with  
170 automated milking system (Lely Astronaut A3, Lely Industries N.V., Maassluis, the Netherlands).  
171 The milk yield was recorded for every milking. The milk samples were collected on 4 consecutive  
172 milkings at 1, 2, 4, 6 and 8 wk after parturition, mixed with preservative (Bronopol, Valio Ltd,



173 Helsinki, Finland), and sent to a commercial laboratory (Valio Ltd., Seinäjoki, Finland) for analysis  
174 of fat, protein, lactose, and urea (MilkoScan 133B analyzer; Foss Electric A/S, Hillerød, Denmark).

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176 The cows were weighed on 2 consecutive days at 8, 6, 4, 2 and 1 wk before the expected calving  
177 day, on d 1 and d 2 postpartum, and at 1, 2, 4, 6 and 8 wk after calving. The cows were always  
178 weighed at the same time of day, starting at 1300 h, to minimize the influence of milking and  
179 feeding. In the case of postdate pregnancies, additional weighing was done on alternate days until  
180 the due date. Body condition score (Edmonson et al., 1989) was recorded by the same person  
181 throughout the experiment at the same time points as weighing. The cross section of the longissimus  
182 dorsi muscle (pars lumbalis) and the subcutaneous fat thickness were measured on the right  
183 transversal process of the third lumbar vertebra, 2 to 3 cm medially from the lateral end at 14 days  
184 prior to, and at 1, 7 and 28 d after parturition using an Aloka SSD-500 (Aloka, Tokyo, Japan)  
185 ultrasound scanner with a 5.0 MHz transducer after shaving of the positions.

186

#### 187 ***Blood Samples and Intravenous Glucose Tolerance Tests***

188 Blood sampling was performed by puncture of the coccygeal blood vessels at 56, 42, 28, 21, 16, 12,  
189 7, 5, 3 and 1 d before the expected calving date and at 1, 3, 5, 7, 14, 21, 28, 42 and 56 d after calving.  
190 The samples were collected into evacuated collection tubes (Vacutainer; BD Medical, New Jersey,  
191 USA) containing potassium ethylene diamine tetra-acetic acid (EDTA) and placed on ice. Blood  
192 samples were centrifuged at 2,220 x g for 10 min to separate plasma, which was then stored at -20  
193 °C for analyses of glucose, insulin, glucagon,  $\beta$ -hydroxybutyrate (**BHB**), NEFA, and glycerol.  
194 Plasma samples for 3-methylhistidine (3-MH) were collected at 12 days prior to the expected  
195 calving, and at 1, 7 and 28 d after parturition and handled as described above. The samples were  
196 precipitated with 10% sulfosalicylic acid and analyzed by UPLC (Acquity UPLC, Waters, USA)

equipped with BEH C18 column (100mm × 2.1mm) according to manufacturer's instructions. Plasma glycerol was analyzed with a direct colorimetric method (Foster et al., 1978) using a commercially available kit GY105 (Randox Laboratories Limited, Crumlin, United Kingdom). Plasma glucose, NEFA and insulin were analyzed as described by Salin et al. (2012) and BHB, 3-MH and glucagon as described by Kokkonen et al. (2018). Intra-assay and interassay CVs for plasma metabolites and hormones, liver TG and total lipids, hepatic, and AT gene expression of enzymes involved in the gluconeogenesis, and fatty acid metabolism were reported by Selim et al. (2015).

Intravenous glucose tolerance tests (IVGTT) were performed  $13 \pm 5$  d prior to the actual delivery date and  $9 \pm 1$  d postpartum at 0900 h, as described in detail by Salin et al. (2012). Briefly, on the previous day sterile indwelling catheters [left: Mila 14G (Mila International, Kentucky, USA); right: homemade catheter made of silicone tubing] were inserted into jugular veins and sutured to the skin (Vetafil Bengen, Hannover, Germany), and a 25-cm polyvinyl chloride elongation tube (Connecta, BD Medical, Franklin Lakes, NJ) was connected to the catheters. Left catheter was used for glucose infusion and right one for collection of blood samples. Infusion of 0.25 g of glucose/kg of BW (Glucos. 300 mg/mL, B.Braun Melsungen AG, Germany) was performed over  $4.5 \pm 1.4$  min, and  $4.6 \pm 2.3$  min (mean  $\pm$  SD) pre- and postpartum, respectively with an average infusion rate of  $151 \pm 39$  mL of glucose solution/min. Blood samples were collected via catheters at -10, -5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210 and 240 min relative to the initiation of glucose infusion. The samples were handled as described above. Feed but not water was withheld 2 hours before and during the IVGTT.

### ***Calculations and Statistical Analyses***

One cow from CEI was excluded from the trial 4 wk after parturition due to presumed abomasal problems. The data for one cow in HEI was omitted from the statistical calculations for the first 2

222 wk of the dry period due to error in treatment allocation. Similarly, the data of one cow in CEI  
223 treatment displayed abnormal NEFA response to glucose bolus prepartum, hence the data set of the  
224 particular animal was considered outlier and excluded from the analysis of NEFA dynamics during  
225 the IVGTT at 13 d prior to parturition.

226  
227 The metabolizable energy concentration of the forages were calculated based on the digestible OM  
228 concentration in DM (D-value) and the ME concentration of concentrates based on the feed tables  
229 (Luke, 2017). The energy balance (EB) was calculated as the difference between the ME intake and  
230 the ME requirements (Luke, 2017). A correction equation for associative effects of feeds and feeding  
231 level was used when ME intake was calculated (Luke, 2017). The ECM was calculated according  
232 to Sjaunja et al. (1991).

233  
234 The net incremental area under the response curve (AUC) for plasma glucose, insulin and NEFA  
235 during the first 60 and 240 min of IVGTT was calculated as described by Salin et al. (2017). The  
236 decrement of NEFA was calculated by subtracting the nadir concentration from the basal NEFA  
237 concentration. Clearance rate (CR; %/min) of metabolites and insulin were calculated using PROC  
238 NLIN of SAS (version 9.3) as described previously (Pires et al., 2007; Salin et al., 2012). For each  
239 IVGTT, the Minimal Model (**MM**; Bergman, 1989) was applied to glucose and insulin curves using  
240 a commercial software (MinMod Millenium, MINMOD Inc., Pasadena, CA) with methods described  
241 previously (Boston et al., 2003; Salin et al., 2012). Briefly, the model provides values for insulin  
242 sensitivity (**SI**;  $\times 10^{-4} \text{ min}^{-1}/\mu\text{IU/mL}$ ), defined as the fractional rate of glucose uptake per unit of  
243 plasma insulin; glucose effectiveness (**Sg**;  $\text{min}^{-1}$ ), characterizes non-insulin mediated glucose  
244 disposal; acute insulin response to glucose (**AIR<sub>G</sub>**;  $\mu\text{IU/mL/min}$ ), representing the endogenous  
245 insulin response to a glucose bolus; and disposition index (**DI**), a measure of overall glucose  
246 tolerance, and insulin responsiveness corrected for changes in SI. DI is calculated as the product of

247 SI and AIR<sub>G</sub>. For the evaluation of the NEFA dynamics during the IVGTT, a NEFA model was used  
248 (Boston and Moate, 2008).

249

250 Data for feed intake, milk production and blood hormone and metabolite concentrations, BW, BCS,  
251 EB, and postpartal plasma 3-MH and back muscle diameter were analyzed as repeated measures  
252 ANOVA using the MIXED procedure of SAS version 9.3 (SAS Institute, Cary, NC, USA).  
253 Prepartum and postpartum data were analyzed separately. Measurements of DMI, EB, and milk  
254 production were reduced to weekly means before statistical analysis. Three covariance structures  
255 were tested for each variable analyzed; compound symmetry (CS), unstructured (UN) and  
256 autoregressive order 1 [AR(1)]. For unequally spaced measures, spatial power [SP(POW)] was used  
257 instead of AR(1). The statistical model included fixed effects of treatment, time (day or week relative  
258 to calving), the interaction between treatment and time (diet x time), and a random effect of block  
259 and interaction between block and time. Degrees of freedom were estimated by using the Kenward-  
260 Roger option in the model statement. The covariance structure that resulted in the smallest  
261 Schwarz's Bayesian information criterion was used.

262

263 The data of BW, BCS and EB at particular time points and their changes, prepartal plasma 3-MH and  
264 back muscle diameter, as well as data derived from the IVGTT were analyzed by ANOVA with a  
265 model including fixed effect of treatment and random effect of block using the PROC MIXED of  
266 SAS (version 9.3; SAS Institute Inc., Cary, NC). A fixed effect of interval between the sampling day  
267 and the actual day of parturition was included in the statistical model of the prepartal IVGTT data.

268

269 Prior to statistical analysis, residuals of each variable were checked for normality using the MIXED  
270 and UNIVARIATE procedures of SAS. To correct for the deviations from normality and  
271 homoscedasticity of the residuals for the data of each variable, the variables were subjected to

reciprocal or log-transformation (LN), when needed. The Friedman's nonparametric test was used whenever the above mentioned normalization processes were not effective, as was the case in the MM derived prepartal estimates SI and Sg. All values in the tables and figures are reported as least squares means and standard error of the mean (SEM). Back-transformed least squares means, and SEM are reported from the analysis of transformed data. The relationships between plasma concentrations and calculated parameters describing insulin sensitivity during IVGTT were investigated by Spearman's correlation analysis, using the CORR procedure of SAS. The effects were considered statistically significant at  $P < 0.05$ , and trends for effects are discussed at  $0.05 \leq P < 0.10$ .

280

## 281 **RESULTS**

282

### 283 ***Diets and Intakes***

284 The analyzed composition of prepartum and postpartum diets is shown in Table 1. The formulation of TMR was successful and the targeted difference in the NDF-content of the diets between HEI and CEI was achieved. The average NDF-content of the GS of HEI was lower than the NDF levels measured from the preliminary samples, leading to an average NDF difference of 120 g/kg of DM between the treatments. The inclusion of rapeseed meal in the TMR was successful in balancing the CP content of CEI close to the CP content of GS fed in HEI. The digestible OM content (D-value) of the CEI was, as expected, lower than that of HEI.

291

292 The experimental design led to expected significant differences in DMI and intakes of other nutrients as well as in ME-intakes between the treatments during the prepartum period (Table 2). The total DM and forage DM intake (Figure 1) of HEI was 2.2 and 2.9 kg/d higher in HEI than in CEI during the dry period, respectively ( $P < 0.01$ ). The DMI expressed as % of BW tended to be greater during the entire dry period in HEI than in CEI ( $P = 0.05$ ) and the significant diet x time interaction showed that

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the difference in DMI was largest on wk -8 and -6 relative to parturition, averaging 2.0% and 1.5% of BW (SEM 0.12; P = 0.04). The EB during the dry period (Figure 2) was approximately 30 % greater in HEI than in CEI (P < 0.001) and the average energy intake was 141% and 108 % (SEM 5.46; P < 0.001) of the requirements (Luke, 2017) in HEI and in CEI, respectively. After parturition, the dry period feeding had no effect on total DMI and intakes of nutrients except for a significant difference in concentrate DMI. The HEI cows had higher intake of concentrates than CEI cows from wk 4 onwards (diet x time P = 0.05), resulting in 0.4 kg/d greater postpartal average DMI of concentrates in HEI than in CEI (SEM 0.09; P = 0.003). The energy balance was not affected by the prepartal dietary treatment after the parturition (P = 0.46).

306

### 307 ***Body Reserves***

The cows were dry for an average of  $67 \pm 11.1$  d (mean  $\pm$  SD). The parameters describing the changes of body reserves are shown in Table 3. The initial BW and BCS were not different between treatments, by design. However, the significant diet x time interactions in BW (P = 0.005) and BCS (P = 0.02) during the dry period indicated steeper increment of BW and BCS in HEI than in CEI (Supplemental figure S1 and S2). The BW change from d -56 to d -5 prior to parturition was greater in HEI than in CEI (P = 0.03) and accounted for a total increment of 75.0 vs. 40.8 kg (SEM 9.1), respectively. The BCS change tended to be greater in HEI than in CEI prepartum and increased in total 0.37 vs. 0.14 (SEM 0.08; P = 0.07) from d -56 to d -5. The birth weights of calves did not differ between the treatments. We found no treatment and no diet x time interaction effects on either BW, BCS or changes in these parameters during the early lactation. Back muscle diameter on -14 d prepartum was similar in both groups but declined to a smaller average value in CEI than in HEI during the first 4 wk of lactation (P = 0.01). We observed no differences in back fat thickness.

320

### 321 ***Plasma Metabolites and Hormone Concentrations***

Basal plasma hormone and metabolite concentrations are depicted in Table 4. The concentration of plasma glucose before parturition was higher in HEI than in CEI, and the difference was most evident during the last 2 wk of pregnancy (diet x time,  $P = 0.06$ ; Figure 3). After parturition, the HEI cows tended to have higher glucose concentrations than CEI cows ( $P = 0.09$ ). We found a tendency for greater insulin levels in HEI than in CEI during the dry period ( $P = 0.07$ ), the difference being most pronounced during the last 4 wk prior to parturition (diet x time,  $P = 0.08$ ; Figure 4). The insulin concentration did not differ between treatments after parturition. We did not observe a difference in either the plasma glucagon concentration or in the glucagon to insulin ratio during the experimental period. The average plasma concentration of NEFA did not differ prepartum, but the rise of NEFA concentration during the last week of pregnancy tended to be steeper in CEI than in HEI (diet x time,  $P = 0.07$ ; Figure 5). After parturition, there were no differences in the average plasma NEFA concentration. Plasma BHB concentrations were greater in HEI than in CEI during the dry period ( $P = 0.006$ ; Figure 6). After calving, the BHB levels increased until 2 wk and 4 wk of lactation in HEI and CEI, respectively. CEI cows had higher BHB at wk 6 and 8 (diet x time,  $P = 0.07$ ). Plasma glycerol concentrations were not affected by the prepartal dietary treatments. Plasma 3-MH tended to be higher in CEI than in HEI on -12 d prepartum ( $P = 0.09$ ), while no differences in plasma 3-MH were found after parturition.

339

#### 340 ***Milk Production and Composition***

The milk production parameters are depicted in Table 5. The average milk yield and the ECM were not affected by the treatments. However, a significant diet x time interaction was observed, indicating higher milk yield in HEI than CEI from week 5 onwards, the average difference being  $5.3 \pm 1.6$  kg on wk 7 and 8 ( $P = 0.007$ ; Supplemental figure S3). The feed efficiency (ECM/kg DMI) was not affected by prepartal diet. Increase in feed efficiency tended to be greater in HEI than in CEI during the first 2 wk of lactation, the difference was most pronounced in wk 2 (diet x time,  $P = 0.07$ ). Milk

fat-% was greater in HEI vs. CEI on lactation wk 1, 2, and 6 (diet x time,  $P = 0.003$ ). Milk protein and lactose concentrations were not different between treatments. Milk urea tended to be higher in HEI than in CEI over time, the difference being most pronounced on lactation wk 4 (diet x time,  $P = 0.09$ ). The fat yield was not different between treatments. The protein yield tended to be higher in HEI vs. CEI from wk 6 onwards, the difference being greatest on wk 8 (diet x time,  $P = 0.05$ ). The lactose yield was higher in HEI than in CEI on wk 8 (diet x time,  $P = 0.01$ ).

353

#### 354 ***Glucose, Insulin, and NEFA Dynamics During the IVGTT***

Basal and peak concentrations, and CR of glucose prepartum were not affected by the dietary treatments (Table 6). However, glucose area under the response curve was smaller in HEI than in CEI during the prepartal IVGTT ( $AUC_{240}$ ;  $P = 0.02$ ; Figure 7A). Prepartal diet affected the endogenous insulin response to glucose infusion during the IVGTT at 13 d prior to parturition (Figure 7B). The peak concentration ( $P = 0.01$ ) and the AUC of insulin during the first hour of the IVGTT ( $P = 0.03$ ) were greater in HEI than in CEI, and the AUC during the 240 min IVGTT tended to be greater in HEI than in CEI ( $P = 0.05$ ). The effect of the interval between the IVGTT and the day of calving was significant during the prepartal IVGTT for insulin peak concentration and insulin AUC ( $P < 0.05$ ), indicating that cows closer to their due date had smaller insulin AUC and lower peak concentrations. The basal and nadir concentrations of NEFA, as well as CR and AUC did not differ between treatments pre- and postpartum (Figure 7C). However, during the prepartal IVGTT the NEFA Model derived parameter for latency was greater ( $P = 0.04$ ) and FFA0 tended to be smaller ( $P = 0.09$ ) in HEI than in CEI.

368

The dry period dietary treatment had minor effect on MM estimates, as the only significant difference was higher AIRg in HEI than in CEI ( $P = 0.002$ ) during the prepartal IVGTT (Table 6). The effect of the interval between the IVGTT and the day of calving was significant during the prepartal IVGTT



372 for DI ( $P = 0.03$ ), showing that the closer the due date the lower the DI. The values of Sg, SI and DI  
373 did not differ between the treatments at either time points ( $P > 0.10$ ).

374

375 We found no treatment differences in glucose, insulin and NEFA responses to infusion of glucose  
376 after parturition ( $P > 0.10$ ; Figure 8). The basal NEFA had a strong positive correlation with NEFA  
377 decrement both in prepartal ( $r = 0.98$ ;  $P < 0.001$ ) and postpartal ( $r = 0.74$ ;  $P < 0.001$ ) IVGTT.  
378 Similarly, basal NEFA had a negative association with NEFA AUC<sub>60</sub> both in prepartal ( $r = -0.69$ ;  $P$   
379  $< 0.004$ ) and in postpartal IVGTT ( $r = -0.50$ ;  $P = 0.049$ ).

380

381

## DISCUSSION

382 The main objective of this study was to investigate whether controlling energy intake of dairy cows  
383 by diluting a moderately digestible GS with WS during the 8 wk dry period affects whole body IR,  
384 and tissue deposition and mobilization during the early lactation in comparison to ad libitum access  
385 of GS. We assessed these conditions by measuring the changes of plasma metabolite and hormone  
386 concentrations under basal and stimulated conditions, as well as by investigating the changes on  
387 BCS and BW, and on production performance of early lactation. Given that GS is the main source  
388 of forage for dairy cows in many Northern European countries during the indoor feeding period, we  
389 have previously assessed the effects of different allowances of sole GS during the dry period on  
390 metabolic and production responses (Salin et al. 2017; Kokkonen et al., 2018). In the present  
391 experiment, GS as a sole forage source, and GS diluted with straw fed ad libitum were chosen for  
392 comparison to investigate a more practical feeding regime for loose housing systems.

393

394 *Dry matter intake*

395 The prepartal DMI was well maintained in CEI cows in accordance to earlier studies showing that  
396 controlling of energy intake either by restriction of the amount of TMR or GS (Agenäs et al., 2003;  
397 Kokkonen et al., 2018) or the composition of the diet (Rabelo et al., 2003; Vickers et al., 2013)  
398 prevented the decline in DMI during the last weeks of pregnancy. The moderate decline in prepartal  
399 DMI of HEI cows during the last 4 wk prepartum in the current study ( $0.9 \pm 0.73$  kg/d) is in  
400 agreement with Agenäs et al., (2003) who reported an average of 1 kg/d decline in DMI during the  
401 same time period in overfed cows on GS-based diets. In comparison, the DMI decline in cows fed  
402 ad libitum GS with or without barley straw in a series of experiments during the final 5 wk of the dry  
403 period (NDF range 452 to 689 g/kg DM) was in average 1.47 kg/day, half of which occurred during  
404 the week preceding calving (Dewhurst et al., 2010). Although we did not investigate digestibility of  
405 the nutrients in this study, the lower intake of CEI is at least partly attributable to the lower nutrient  
406 digestibility resulting from inherent characteristics of straw fiber (Dewhurst et al., 2000).

407

408 We did not find any difference in postpartal DMI in agreement with other studies with moderate (30  
409 to 40 %) overfeeding of energy during the dry period (Butler et al., 2011; Mann et al., 2015;  
410 Kokkonen et al., 2018). These results contrast with previous studies showing that high energy diets  
411 (150 to 160% of energy requirement) during late pregnancy resulted in lower feed intake during the  
412 early lactation in MS-based diets (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley,  
413 2010). The moderately, but statistically significantly lower concentrate DMI in CEI than HEI after  
414 parturition, especially during the second month of lactation, could possibly be a carry-over effect of  
415 the prepartal TMR. The inclusion of WS into diet of CEI may have affected rumen adaptation  
416 mechanism, e.g. papillae development and structural changes (Odongo et al., 2006; Steele et al.,  
417 2011). The very moderate amount of concentrates given for only  $12 \pm 5$  d (mean  $\pm$  SD) prepartum  
418 may have resulted in a too low non-structural carbohydrate (NSC) intake of CEI in order to optimize  
419 the adaptation to a concentrate rich lactation ration after parturition. In fact, higher contents of

420 digestible carbohydrate, such as starch, enhanced the overall VFA absorption capacity via rumen  
421 papillae development (Goodlad, 1981; Dirksen et al., 1985).

422

### 423 ***Plasma Metabolites and Hormones***

424 Higher prepartal concentration of basal glucose in cows fed high energy diets compared with  
425 controlled energy diets during the entire 8 wk dry period is analogous with earlier research (Douglas  
426 et al., 2006; Janovick et al., 2011; Mann et al., 2016), whereas we did not observe a corresponding  
427 difference when different amounts of GS were fed to dry cows during the 6 wk dry period  
428 (Kokkonen et al., 2018). The higher blood glucose concentration in HEI is most likely a result of  
429 increased availability of propionate for hepatic gluconeogenesis (Janovick et al., 2011; Mann et al.,  
430 2016) as the TMR with high amounts of straw lacks precursors for gluconeogenesis. Correspondent  
431 with higher glucose levels, the HEI cows tended to have higher insulin concentration during the  
432 prepartal period, analogous to other recent studies showing that energy overfeeding during the dry  
433 period resulted in higher prepartum insulin concentrations compared with cows fed restricted energy  
434 (Holtenius et al., 2003; Douglas et al., 2006; Janovick et al., 2011). The higher, but still moderate  
435 plasma BHB in HEI than in CEI cows prepartum is most likely a consequence of a greater ruminal  
436 butyrate production due to higher intake of GS, and not an indicator of metabolic imbalance (Roche  
437 et al., 2013). In line with this, the mRNA expression of mitochondrial CPT1 activity in the liver at  
438 d -14, 1 and 7 d relative to parturition was not changed in the current animals (Selim et al., 2015)  
439 suggesting that the translocation of FA derivatives to hepatic mitochondrial beta-oxidation was  
440 unchanged around parturition. The results agree with earlier studies reporting lower prepartal BHB  
441 in cows on controlled energy diets than in cows on higher energy diets with a normal or low BCS at  
442 dry-off (Mann et al., 2015; Little et al., 2016). Butler et al., (2011) showed greater blood  
443 concentrations of BHB in GS fed cows than in cows fed a wheat-based TMR at 1 wk before

parturition and during the first 4 weeks postpartum without any treatment effect on incidence of ketosis.

The tendency for higher plasma glucose in HEI after parturition is inconsistent with the absence of differences in plasma glucagon concentration or glucagon to insulin ration suggesting no differences in gluconeogenesis from amino acids and lactate (Aschenbach et al. 2010). The higher plasma glucose is also inconsistent with the results of Selim et al. (2015) from the same cows suggesting attenuated increase of hepatic gluconeogenic activity from propionate in HEI, as the hepatic PCK1 mRNA expression was downregulated in HEI but not in CEI at d 1 and d 7 of lactation when compared with d -8. The higher BHB concentration in CEI than HEI at d 42 and 56 indicates compensation of insufficient supply of glucose precursors, as suggested by lower concentrate DMI. The higher BHB concentration may have also served as tissue protective mechanism in CEI, because BHB has been shown to inhibit basal AT lipolysis in vitro in a dose-dependent matter (Van der Drift et al., 2013). Thus, the feed-back mechanisms may have prevented toxic effects of high BHB levels on tissues by shutting down further release of additional NEFA (Rukkwamsuk et al., 1998). This may explain the lack of differences in NEFA concentrations between CEI and HEI at d 42 and 56.

#### ***Indicators of Tissue Accretion and Mobilization***

The cows in HEI gained more BW during the 8 wk dry period. Although the difference in BW gain between energy levels was greater in the present experiment than in our previous experiment (Kokkonen et al. 2018), we expected a larger response of surplus EI on BW and BCS gain before calving. According to the Finnish nutrient requirements (Luke 2017) an average ME-difference of 34 MJ/d between the treatments during the dry period should have resulted in an approximate difference of 1 kg/d in daily BW change instead of reported value of 0.6 kg/d. Thus, our findings

468 support recent studies postulating that ME requirements of pregnant cows may be underestimated in  
469 different energy calculation systems, and that the actual requirements for maintenance and lactation  
470 may be larger than reported (Mandok et al., 2013; Kokkonen and Vanhatalo, 2014). Moreover, it  
471 seems that cows in a fairly good body condition at dry-off, as were all the cows in our study, do not  
472 gain a lot of BCS (0.3 units in average) in GS-based feeding during an 8 wk dry period in agreement  
473 with our recent study with a shorter dry period of 6 wk (Kokkonen et al., 2018). However, higher  
474 prepartal energy intake affected the onset of excessive tissue mobilization near calving. The tendency  
475 for a more pronounced rise of plasma NEFA and higher plasma 3-MH in CEI in the weeks preceding  
476 calving indicates that CEI cows initiated the mobilization process at an earlier stage precalving.

477

478 In contrast to studies showing that postpartal BW losses and NEFA concentrations were lower when  
479 dietary energy intake was restricted prepartum (reflecting positive effect of dry period feeding level  
480 on energy balance postpartum) in TMR fed dairy cows (Douglas et al., 2006; Janovick and Drackley  
481 2010; Janovick et al., 2011), we did not find any differences neither in BW and BCS losses nor in  
482 plasma NEFA concentrations after parturition. Our data suggest that cows on GS-based diets pre-  
483 and postpartum are not prone to large changes in BCS and BW after parturition, concordant with  
484 studies on similar forage type (Agenäs et al., 2003; Vickers et al., 2013; Little et al., 2016; Kokkonen  
485 et al., 2018). More pronounced differences in prepartal BW changes on GS or MS diets have resulted  
486 in increased AT mobilization after parturition (Kokkonen et al., 2005; Douglas et al., 2006). Our  
487 results suggest that overfeeding on GS-based diets has only moderate effect on metabolic flexibility  
488 of transition dairy cows. Conversely, a recent review underlined negative effects of over-  
489 conditioning on MS-based diets with excessive AT mobilization on metabolic disorders of cows in  
490 early lactation (Drackley and Cardoso, 2014). The observed strong positive correlation of initial BCS  
491 and that of the BCS at 5 d prior to parturition is in agreement with Kokkonen et al. (2018). These  
492 findings support the earlier suggestions (Friggens et al., 2004) implying that dairy cows have an

493 inherent level of body reserves towards which their metabolism is aiming for during the late lactation  
494 and in the transition period in order to restore the genetically predestined body condition. Further,  
495 the absence of differences in BCS at calving or in the mobilization of body tissues after parturition  
496 imply that neither the BCS at calving (as suggested by e.g. Roche et al., 2009; 2015; Pires et al.,  
497 2013) nor the prepartal energy intake per se were determinants of the postpartal performance after  
498 parturition in the current study.

499

### 500 ***Production Responses***

501 The milk yield was moderately affected by prepartal feeding, as we observed greater milk yield in  
502 HEI than CEI from wk 4 onwards. Earlier studies comparing GS and mixture of GS and straw  
503 prepartum reported increased milk yield with GS (Dewhurst et al., 2000; McNamara et al., 2003;  
504 Ryan et al., 2003). However, those studies showed that the greatest influence on milk production  
505 performance was observed during the early weeks after calving. This probably resulted from  
506 improved BCS at calving on GS, because the initial BCS at beginning of the dry period feeding was  
507 below 2.75 (McNamara et al., 2003; Ryan et al., 2003). Apparently, an identical temporal effect on  
508 the milk yield with the current one was found in our former experiment (Kokkonen et al., 2018)  
509 conducted at the same institute with genetically similar animals from the same herd, fed with  
510 different amounts of GS during the dry period. However, as opposed to current results, the cows on  
511 controlled energy diet cows tended to produce more milk in the earlier study (Kokkonen et al., 2018).  
512 A common element of these two studies was a slightly lower intake of concentrates during the early  
513 lactation in those animals producing less milk after the first month. In the present study, it seems  
514 that a large proportion of straw in the dry period diet or the short concentrate feeding period, or both,  
515 had a negative effect on adaptation of rumen to postcalving diets and subsequent production  
516 responses, as discussed earlier in this paper. With a moderately digestible GS, there is no need to  
517 dilute the feed composition by adding of straw, as this dry period feeding practice had moderate

negative carry-over effects on postpartal concentrate intake and consequently on early lactation performance in multiparous cows of good body condition at calving. The lack of treatment effects on milk fat and protein contents is in line with earlier experiments on GS-based diets (Agenäs et al., 2003; Kokkonen et al., 2018) reflecting the absence of any obvious differences in energy balance and lipid mobilization during the early lactation. This also indicates that early lactating cows have a potential to compensate for changes in prepartal nutrient intake providing that the cows receive a standard lactation ration of high quality (Agenäs et al., 2003).

#### ***IVGTT***

The observed greater insulin response of HEI cows before calving (greater AUC of insulin and AIR<sub>g</sub>) and the subsequent smaller AUC of glucose suggest that glucose tolerance of HEI was preserved before parturition. However, any environmental change in insulin sensitivity, for instance in response to obesity, will be compensated by an increase in insulin secretion in response to glucose (Bergman, 1989; Kahn et al., 1993). Given the former, we may speculate that the greater insulin secretion in HEI was a compensatory mechanism in response to reduced insulin sensitivity of the peripheral tissues to preserve the glucose tolerance. Salin et al. (2017) found no effect of higher energy intake on insulin response when comparing different GS allowances during the dry period. Similarly, overfeeding of energy in the close-up dry period alone or during the entire dry period did not affect insulin response during IVGTT in cows on TMR based on MS plus WS (Schoenberg et al., 2012; Mann et al., 2016). In contrast, Jaakson et al., (2018) reported higher insulin response to IVGTT at -21 d in cows with BCS > 3.75 (1-5 scale) when compared with thin cows (BCS < 3.0) on GS and hay based TMR. However, as opposed to current results, the over-conditioned cows had larger glucose AUC than the thinner cows, indicating a higher degree of IR. The discrepancies between studies may stem from different timing of the challenges, and from differences in feed composition, breed, and from dissimilarities in initial and achieved BCS between treatments.

543

544 In agreement with Salin et al. (2017) reporting attenuated NEFA response of overfed cows during  
545 the prepartal IVGTT and enhanced sensitivity of AT after parturition, we found indications of dietary  
546 effects on insulin's action on inhibition of lipolysis on GS-based diets. However, the effects in this  
547 study were evident only before parturition, as the HEI cows needed greater insulin concentrations to  
548 elicit a similar NEFA response than CEI cows in prepartal IVGTT. The former may reflect reduced  
549 AT sensitivity to insulin in response to overfeeding. The greater latency of NEFA response during  
550 the prepartal IVGTT in HEI than in CEI reinforces the suggestion that insulin sensitivity in AT of  
551 HEI was affected by dry period energy intake. The latency is thought to result from the time it takes  
552 for the challenge to trigger the suppression of lipolysis (Boston and Moate, 2008). The extended  
553 latency period of HEI cows in prepartal IVGTT, together with the higher insulin response and a  
554 similar eventual NEFA suppression, may insinuate that the antilipolytic action of insulin was  
555 compromised in cows with higher BW gain during the dry period. Similarly, cows that were losing  
556 high levels of BW had more refractory AT to insulin both pre and postpartum (Zachut et al., 2013).  
557 By contrast, Mann et al., (2016) did not find any effect of different energy intake on NEFA response  
558 during the transition period, neither did Marett et al., (2015) during different stages of lactation.  
559 Insulin response of the glucose metabolism, but not that of fatty acid metabolism, was negatively  
560 associated with excessive accumulation of AT in late pregnant dairy cows as assessed by HEC test,  
561 while insulin sensitivity in AT of over conditioned cows with greater adipocytes was preserved in  
562 vitro (De Koster et al., 2015, 2016). Recently, glucose transporter 4 protein synthesis in AT of  
563 overconditioned cows was reduced, suggesting a more severe IR prepartum, while no differences in  
564 insulin signaling potential were found relative to thinner cows (Jaakson et al., 2018).

565

566 We showed that basal NEFA had a strong correlation with NEFA decrement and NEFA AUC during  
567 IVGTT both pre- and postpartum. Analogous results have been reported in studies where insulin



568 sensitivity was assessed by different methods (Patton et al., 2009; Schoenberg et al., 2012; De Koster  
569 et al., 2015; Salin et al., 2017). The results may imply that the NEFA decrement during stimulated  
570 conditions is not only a result of the direct insulin response (the insulin AUC) after a glucose bolus  
571 but is also partially mediated by the secondary effect of insulin and lipolytic agents on basal lipolysis  
572 prior to IVGTT. Indeed, increased fatness of dairy cows amplified the lipolytic response of AT to  
573 catecholamine stimulation (Kokkonen et al., 2005) especially in the dry period (Theilgaard et al.,  
574 2002). Similarly, both the basal and stimulated in vitro AT sensitivity to lipolytic agents were greater  
575 in over-conditioned than in normal conditioned cows before parturition (De Koster et al., 2016a).  
576 Furthermore, in humans at least, the inhibitory action of insulin on AT lipolysis is dependent on the  
577 prevailing lipolytic activity, such that the antilipolytic effect of the hormone is more pronounced  
578 when the rate of lipolysis is augmented, probably due to increased insulin receptor and signal  
579 transduction activity (Zierath et al., 1998). Finally, the shutdown of lipolysis during the IVGTT  
580 challenge of ruminants may also be partly directly regulated by glucose, which is the metabolic driver  
581 of the degree of NEFA suppression in human AT cells (Arner et al., 1983; Qvisth et al., 2004).

582

583 Whilst it was not our main intention to compare insulin responsiveness and sensitivity of tissues at  
584 different time points in the current study, we found that MM derived values of SI and DI for the  
585 IVGTT at -13 d were numerically smaller than at +9 d. When the relationship of SI and AIRg (=  $DI$ ) is visualized as shown in Figure 9, the left and upward shift of the  $DI$  values in HEI at -13 d  
586 relative to parturition underpin that the compensatory insulin secretion to match the decrease in  
587 insulin sensitivity was sufficient. However, as the  $DI$  reflects the ability of the  $\beta$ -cells of the  
588 pancreatic islets to compensate for IR (Bergman, 1989) the very low prepartal values of  $DI$  point to  
589 an insulin insensitive pancreas in response to glucose and to an overall lower compensation for  
590 decreased insulin sensitivity in all animals indicated also by very low SI values in agreement with  
591 earlier studies (Stanley, 2005; De Koster et al., 2016b; Salin et al., 2017). Further, in comparison to  
592

postpartal values across the treatments, the transformation of the values to the right indicate that as the value of SI is greater after parturition there is no need to an additional compensation in insulin secretion indicated by lower DI and AIRg after parturition, in agreement with Salin et al., (2017). Indications of improvement in overall sensitivity of tissues to insulin in early lactation have been published based on different determination methods of IR (Stanley, 2005; Oliveira et al., 2016). Opposing results suggest that (peripheral) insulin sensitivity of dairy cows is not profoundly changed during the transition period, regardless of prepartal feeding and degree of body fat mobilization (Mann et al., 2016; de Koster et al., 2016a; Weber et al., 2016). Varied results from range of different methods of studying IR in cows in late pregnancy and early lactation clearly highlight the challenges in investigation of the transition period metabolism (Marett et al., 2015; De Koster et al., 2017). Additional research is needed to elicit a consensus on the applicability of the Minimal Model in periparturient dairy cows.

Overall, as our previous experiment showed, that when the dry period was relatively short, and the oversupply of energy on GS-based diets was gradually decreased in the close-up dry period, the effects of overfeeding on whole body insulin sensitivity were only evident in the level of AT (Salin et al., 2017). By contrast, GS fed ad libitum for a longer period of 8 wk, as in the present study, generating a more positive EB in dry cows, did not only induce a delayed response to insulin in AT, but also increased insulin secretion in response to IVGTT near parturition. This, in turn accelerated plasma glucose disappearance and inhibited glucose output or both, preserving peripheral glucose tolerance. Not only the difference in energy intake, but also the greater potential of GS to supply glucogenic precursors in comparison to the mixture of GS and WS contributed to the observed effects on glucose and NEFA dynamics orchestrated via insulin.

## CONCLUSIONS

618

619 Overfeeding energy in grass silage-based diet resulted in elevated BW and BCS gain during the dry  
620 period. However, given the average difference of 40% in prepartal energy intake between the  
621 treatments, we observed smaller than expected differences in BW gain during the dry period. Contrary  
622 to the hypothesis, high energy intake during the dry period did not affect mobilization of body  
623 reserves and feed intake after calving, whereas milk yield was greater from wk 5 onwards in overfed  
624 cows. Parameters from prepartal IVGTT indicated that overfed cows had more pronounced insulin  
625 response to glucose load and a smaller glucose AUC, reflecting preserved glucose tolerance. Further,  
626 the delayed NEFA response during prepartal IVGTT suggest attenuated inhibition of lipolysis in  
627 response to oversupply of energy. The dietary differences in propionic acid availability leading to  
628 lower prepartal glucose and insulin levels in TMR fed cows most likely contributed to the observed  
629 responses during the IVGTT prepartum. These effects of prepartal feeding on insulin sensitivity did  
630 not carry over to the early lactation. Our results suggest that controlling energy intake of dry cows by  
631 dilution of moderately digestible grass silage by straw is not beneficial for optimally conditioned  
632 cows. In conclusion, ad libitum feeding of moderately digestible grass silage during the dry period  
633 had only transient effects on metabolic adaptation and insulin sensitivity during the transition period.  
634 This feeding regime was more favorable to early lactation performance than ad libitum fed TMR of  
635 grass silage diluted with WS.

636

637

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889 **Table 1.** Chemical composition and calculated energy content of forages and concentrates in dietary treatments CEI<sup>1</sup> and HEI<sup>2</sup>

Item	TMR <sup>3</sup> CEI	Grass silage <sup>4</sup> CEI	Wheat straw <sup>5</sup> CEI	Rapeseed meal <sup>6</sup> CEI	Grass silage <sup>7</sup> HEI	Grass silage <sup>8</sup> Lactation	Concentrate <sup>9</sup>	Protein supplement <sup>10</sup>
DM, g/kg	272	201	813	880	273	288	873	871
Ash, g/kg DM	60	63	74	68	84	85	69	83
CP, g/kg DM	122	146	48	368	129	146	196	288
Ether extract, g/kg DM	-	-	-	57	-	-	50	78
NDF, g/kg DM	651	595	775	273	531	527	211	202
ME, MJ/kg DM	9.1	10.7	6.4	11.4 <sup>11</sup>	10.1	11.0	12.8 <sup>12</sup>	13.0 <sup>12</sup>
D-value, g/kg DM <sup>13</sup>	587	668	457	-	638	687	-	-
MP, g/kg DM	-	80.5	42.0	169 <sup>11</sup>	62.4	67.3	119 <sup>12</sup>	140 <sup>12</sup>

890 <sup>1</sup>CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

891 <sup>2</sup>HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

892 <sup>3</sup>Total mixed ration fed to CEI during the dry period

893 <sup>4</sup>Grass silage in TMR fed to CEI during the dry period. Mean fermentation characteristics: pH 3.9; (% in DM) lactic acid (3.8), acetic acid (1.5),  
894 butyric acid (0.034), propionic acid (0.14), sugars (6.7); (% of total N) ammonium-N (6.7); soluble N (62).

895 <sup>5</sup>Wheat straw in TMR fed to CEI during the dry period.

896 <sup>6</sup>Rapeseed meal in TMR fed to CEI during the dry period

897 <sup>7</sup>Grass silage fed to HEI during the dry period. Mean fermentation characteristics: pH 4.1; (% in DM) lactic acid (3.3), acetic acid (0.70), butyric  
898 acid (0.00), propionic acid (0.12), sugars (1.6); (% of total N) ammonium-N (5.5); soluble N (47).

899 <sup>8</sup>Grass silage fed during lactation. Mean fermentation characteristics: pH 4.4; (% in DM) lactic acid (3.3), acetic acid (1.4), butyric acid (0.20),  
900 propionic acid (0.12), sugars (1.1); (% of total N) ammonium-N (6.8); soluble N (61)

901 <sup>9</sup>Concentrate fed during the last 12 ± 5 d (mean ± SD) of pregnancy and during lactation.

902 <sup>10</sup>Protein supplement fed during lactation.

903 <sup>11</sup>Values adopted from Luke (2017)

904 <sup>12</sup>Values provided by the manufacturer (Raisio Oy Ltd; Raisio, Finland)

905 <sup>13</sup>In vitro digestible organic matter in DM

906

907 **Table 2.** Effect of dry period energy intake and diet composition on DMI and energy balance

Item	CEI <sup>1</sup>	HEI <sup>2</sup>	SEM	P-value	
				Diet	Diet x Time
<b>Prepartum</b>					
Forage DM, kg/d	10.8	13.7	0.46	<0.001	0.74
Concentrate, DM, kg/d <sup>3</sup>	1.3	1.4	0.10	0.77	0.03
Total DM kg/d	12.0	14.2	0.50	0.002	0.64
DMI, % of BW	1.58	1.86	0.10	0.05	0.04
NDF, kg/d	7.5	7.3	0.3	0.70	0.29
MP, kg/d	0.86	0.89	0.03	0.41	0.54
ME, MJ/d	109	144	4.8	<0.001	0.52
ME-balance, MJ/d	8.52	39.4	4.97	0.004	0.51
<b>Postpartum</b>					
Silage DM, kg/d	11.2	11.3	0.54	0.88	0.82
Concentrate DM, kg/d	10.9	11.3	0.09	0.003	0.05
Total DM, kg/d	22.1	22.6	0.57	0.52	0.57
NDF, kg/d	8.17	8.33	0.29	0.71	0.56
MP, kg/d	2.10	2.17	0.41	0.27	0.34
ME, MJ/d	262	270	6.56	0.42	0.57
ME balance, MJ/d	-47.3	-56.4	8.69	0.46	0.25

908 <sup>1</sup>CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d  
909 <sup>2</sup>HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d  
910 <sup>3</sup>Concentrate fed during the last 12 ± 5 d (mean ± SD) of pregnancy  
911

**Table 3.** Effect of dry period energy intake and diet composition on body composition

Item	CEI <sup>1</sup>	HEI <sup>2</sup>	SEM	P-value
<b>Prepartum</b>				
Body condition score (-8 wk)	3.5	3.4	0.20	0.37
Body condition score, (-5 d)	3.7	3.8	0.20	0.78
Body condition score change	0.14	0.37	0.08	0.07
Body weight, kg (-8 wk)	740	725	39.0	0.12
Body weight, kg (-5 d)	781	800	39.3	0.91
Body weight change, kg/d	0.8	1.4	0.16	0.03
Calf weight, kg	41.2	41.0	1.73	0.91
Back fat thickness, mm (-14 d) <sup>3</sup>	3.7	5.4	1.10	0.21
Back muscle diameter, mm (-14 d) <sup>3</sup>	51.3	52.2	2.29	0.65
<b>Postpartum</b>				
Body condition score, +8 wk	2.9	3.1	0.21	0.37
Body weight, kg (+1/2 d)	719	718	39.8	0.98
Body weight, kg (+8 wk)	700	692	18.9	0.50
Body weight change, kg/d	-0.6	-0.8	0.26	0.73
Back fat thickness, mm <sup>3,4</sup>	1.12	1.35	0.262	0.36
	(3.1)	(3.9)		
Change in back fat thickness (mm) <sup>5</sup>	-0.6	-1.0	0.44	0.51
Back muscle diameter, mm <sup>3</sup>	45.3	47.8	2.17	0.01
Change in back muscle diameter (mm) <sup>5</sup>	-9.2	-6.6	1.6	0.28

<sup>1</sup>CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

<sup>2</sup>HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

<sup>3</sup>Measured with ultrasound on m. longissimus dorsi, pars lumbalis

<sup>4</sup>Values after transformation, back-transformed values are given in parenthesis

<sup>5</sup>The change from d -14 to d +28 relative to parturition.

**Table 4.** Effect of dry period energy intake and diet composition on blood hormone and metabolite concentration

Item	CEI <sup>1</sup>	HEI <sup>2</sup>	SEM	<i>P</i> -value	
				Diet	Diet x Time
<b>Prepartum</b>					
Glucose, mmol/l	3.8	4.0	0.06	0.049	0.06
Log Insulin, $\mu$ IU/ml <sup>3</sup>	2.67 (14.4)	3.00 (20.1)	0.151	0.07	0.08
1 / Glucagon, pg/ml <sup>3, 4</sup>	0.0078 (128.2)	0.0071 (140.8)	0.0006	0.45	0.33
Log Glucagon:insulin, mol/mol <sup>3, 4</sup>	-1.00 (0.37)	-1.03 (0.36)	0.126	0.86	0.60
Log Non-esterified fatty acids, mmol/l	-1.93 (0.15)	-2.09 (0.12)	0.097	0.26	0.07
BHB, mmol/l	0.61	0.67	0.018	0.006	0.27
Log Glycerol, $\mu$ mol/L <sup>3, 5</sup>	3.75 (42.5)	3.83 (46.1)	0.055	0.32	0.93
3-MH, $\mu$ mol/l (d -12)	7.48	5.80	0.734	0.09	-
<b>Postpartum</b>					
Glucose, mmol/l	3.2	3.4	0.09	0.09	0.45
Log Insulin, $\mu$ IU/ml	2.12 (8.3)	2.22 (9.2)	0.107	0.52	0.37
1 / Glucagon, pg/ml <sup>3, 6</sup>	0.0069 (144.9)	0.0076 (131.6)	0.0006	0.43	0.37
Log Glucagon:insulin, mol/mol <sup>6</sup>	-0.33 (0.72)	-0.50 (0.61)	0.142	0.40	0.26
Log Non-esterified fatty acids, mmol/L <sup>3</sup>	-1.06 (0.35)	-0.97 (0.38)	0.133	0.62	0.28
1 / BHB, mmol/l	0.76 (1.32)	0.89 (1.12)	0.091	0.32	0.01
Log Glycerol, $\mu$ mol/L <sup>3</sup>	3.89 (48.9)	3.96 (52.5)	0.074	0.48	0.21
3-MH, $\mu$ mol/l <sup>7</sup>	9.28	8.78	0.819	0.68	0.19

<sup>1</sup>CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

<sup>2</sup>HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

<sup>3</sup> Values after transformation, back-transformed values are given in parenthesis

<sup>4</sup> Sampled on d -56, -12, and d -3 relative to parturition

<sup>5</sup> Sampled on d -56, -42, -12, -7, -5, -3, and d -1 relative to parturition

<sup>6</sup> Sampled on d 1, 7, 14, and d 28 relative to parturition

<sup>7</sup> 3-MH = Plasma 3-methylhistidine concentration sampled on d 1, 7, and d 28 relative to parturition

**Table 5.** Effect of dry period energy intake and diet composition on milk production responses

Item	CEI <sup>1</sup>	HEI <sup>2</sup>	SEM	P-value	
				Diet	Diet x Time
Milk, kg/d	40.1	42.8	1.32	0.18	0.007
ECM, kg/d <sup>3</sup>	41.8	44.3	1.21	0.16	0.27
ECM/kg DMI	1.95	2.05	0.08	0.38	0.02
Fat, g/d	1820	1930	61.4	0.22	0.30
Protein, g/d	1300	1380	38.9	0.18	0.05
Lactose, g/d	1770	1830	78.4	0.58	0.01
<b>Milk composition</b>					
Fat, g/kg	47.5	48.3	1.07	0.59	0.003
Protein, g/kg	34.4	34.3	0.51	0.96	0.57
Lactose, g/kg	45.4	45.3	0.29	0.76	0.55
Urea, mg/100 ml	29.0	29.8	1.86	0.72	0.09

<sup>1</sup>CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

<sup>2</sup>HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

<sup>3</sup>Energy corrected milk calculated according to Sjaunja et al. (1990).

**Table 6.** Effect of dry period energy intake and diet composition on plasma glucose, insulin, and non-esterified fatty acids (NEFA) responses to intravenous glucose tolerance test (IVGTT; 0.25 g of glucose/kg of BW) at d 13 prior to parturition and at d 9 postpartum

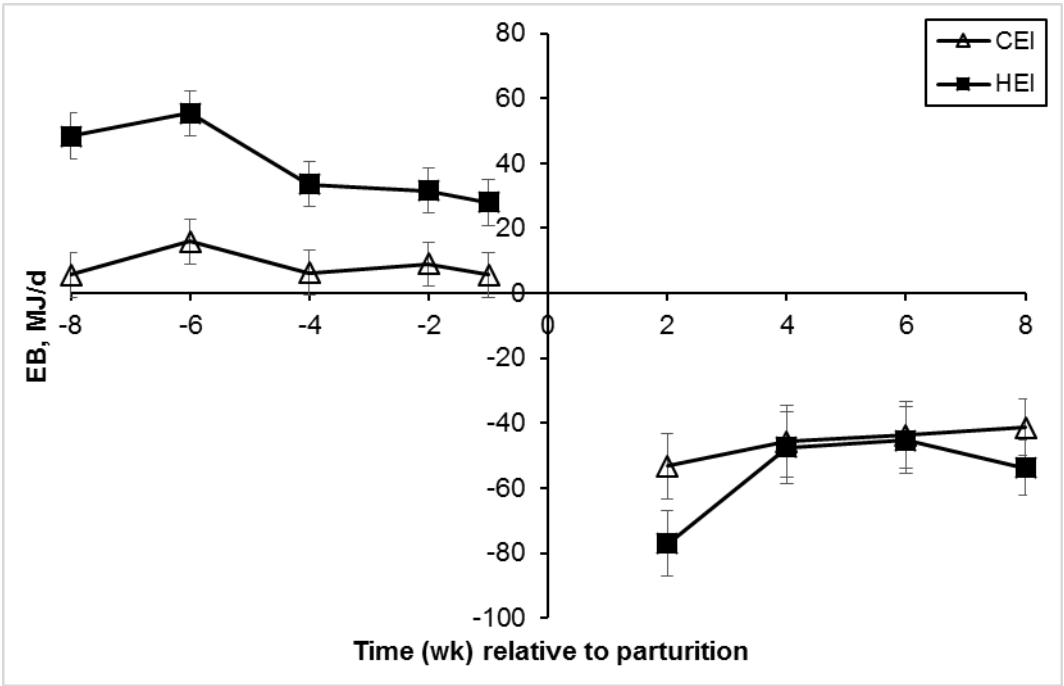
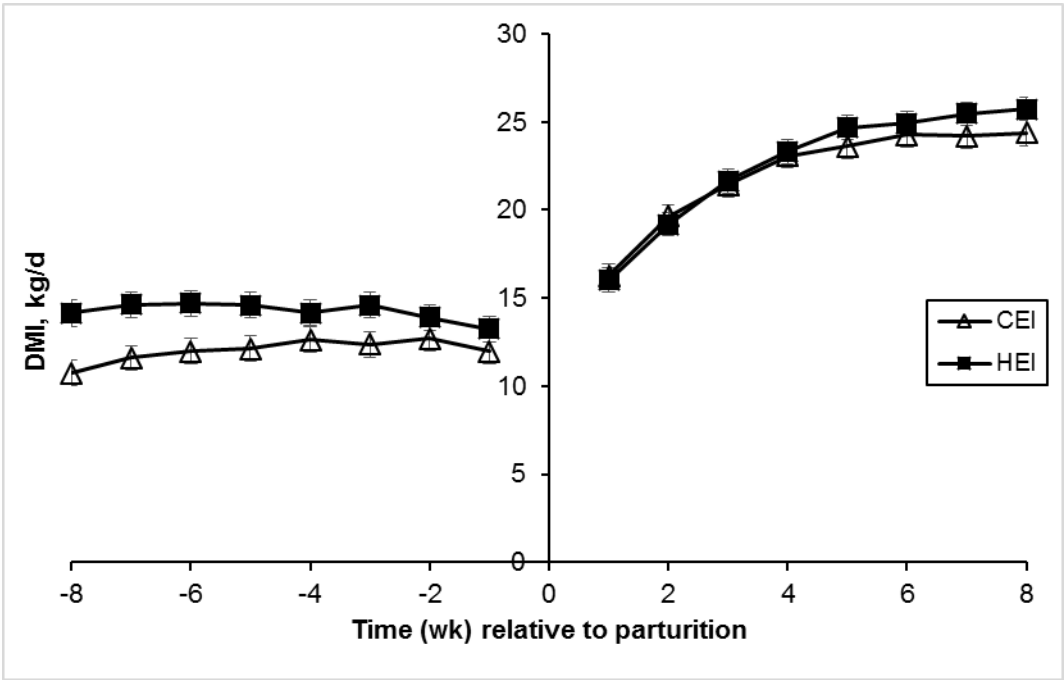
Item <sup>1</sup>	Prepartal IVGTT			<i>P</i>		Postpartal IVGTT			<i>P</i>
	CEI <sup>2</sup>	HEI <sup>3</sup>	SEM	Diet	Day <sup>4</sup>	CEI <sup>2</sup>	HEI <sup>3</sup>	SEM	Diet
	(n = 8) <sup>5</sup>	(n = 8)				(n = 8)	(n = 8)		
<b>Glucose</b>									
Basal (mmol/L)	3.9	4.0	0.12	0.14	0.47	3.0	3.1	0.16	0.51
Peak (mmol/L)	19.4	19.2	0.37	0.71	0.94	15.5	16.8	0.55	0.13
AUC <sub>60</sub> (μIU/mL x 60 min)	404	385	20.0	0.22	0.21	309	294	14.0	0.37
CR <sub>60</sub> (%/min)	1.3	1.4	0.12	0.83	0.93	1.7	2.0	0.17	0.15
AUC <sub>240</sub> (mmol/L x 240 min)	525	413	47.9	0.02	0.08	374	322	30.1	0.22
<b>Insulin</b>									
Basal (μIU/mL)	13.8	15.7	2.03	0.52	0.23	6.2	5.7	0.75	0.64
Peak (μIU/mL)	224	398	73.3	0.02	0.007	110	125	17.5	0.56
CR <sub>60</sub> (%/min)	-0.62	-0.72	0.11	0.40	0.20	0.28	0.21	0.13	0.73
AUC <sub>60</sub> (μIU/mL x 60 min)	7920	13916	2622	0.03	0.01	3185	3844	535	0.55*
AUC <sub>240</sub> (μIU/mL x 240 min)	10064	17762	3520	0.05	0.02	3063	3884	561	0.30
<b>NEFA</b>									
Basal (mmol/L)	0.29	0.24	0.05	0.12	0.03	0.53	0.62	0.07	0.27
Nadir (mmol/L)	0.10	0.08	0.01	0.32	0.045	0.29	0.28	0.03	0.82
NEFA decrement (mmol/L)	0.19	0.16	0.04	0.19	0.09	0.24	0.33	0.05	0.18
CR <sub>60</sub> (%/min)	1.71	1.25	0.48	>0.10**	-	1.2	1.4	0.24	0.45
AUC <sub>60</sub> (μIU/mL x 60 min)	-2.6	-2.3	1.1	0.83	0.70	-5.2	-7.9	2.05	0.38
AUC <sub>240</sub> (mmol/L x 240 min)	-20.0	-13.4	4.33	0.18	0.53	35.0	18.7	17.3	0.53
<b>Minimal model</b>									
SI (x 10 <sup>-4</sup> min <sup>-1</sup> /μIU/mL)	0.67	0.23	0.31	>0.10**	-	1.74	2.28	0.499	0.40
Sg (min <sup>-1</sup> )	0.02	0.03	0.002	>0.10**	-	0.03	0.03	0.002	0.53
AIRg (μIU/mL/min)	695	981	155	0.002	0.57	456	512	70.5	>0.10*
DI	227	241	93.9	0.92	0.03	825	1065	249	0.51
<b>NEFA model</b>									
FFA0 (μmol/L)	390	254	63.5	0.09	0.08	547	641	83.8	0.37
S <sub>FFA</sub> (μmol/L/min)	19.9	21.5	3.59	0.74	0.86	61.7	75.5	13.5	0.49
K <sub>FFA</sub> (/min)	0.03	0.04	0.006	0.13	0.59	0.05	0.05	0.009	0.86
Latency (min)	11.9	16.6	1.26	0.04	0.95	10.5	11.4	1.41	0.66

<sup>1</sup> Basal = average concentration at 10 and 5 min before IVGTT; CR<sub>60</sub> = clearance rate during the first 60 min of IVGTT; AUC<sub>240</sub> = area under the curve during 240 min of IVGTT [(mmol/L for glucose and NEFA, μIU/mL for insulin) x 240 min]; AUC<sub>60</sub> = area under the curve during the first 60 min of IVGTT; NEFA decrement = basal – nadir; SI = insulin sensitivity index; Sg = glucose effectiveness; AIRg = acute insulin response to glucose load; DI = disposition index (= AIRg x SI); FFA0 = basal NEFA estimated by NEFA model analysis; S<sub>FFA</sub> = rate of entry of NEFA to the plasma pool; K<sub>FFA</sub> = rate of removal of NEFA from the plasma pool; Latency = the time until NEFA concentration begin to decline

<sup>2</sup> CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d; <sup>3</sup> HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements

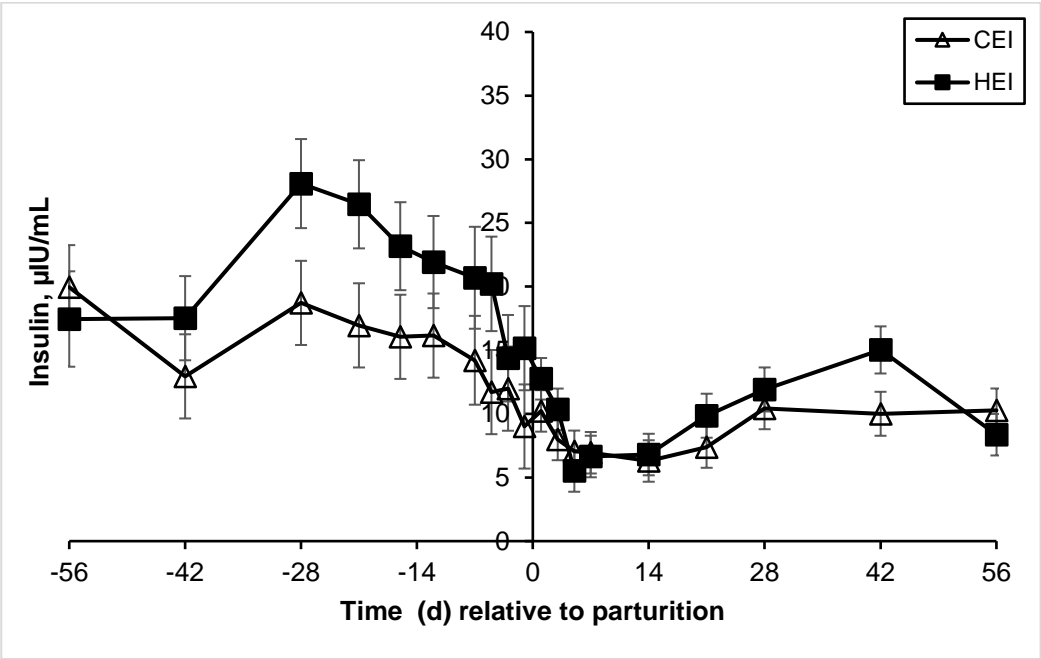
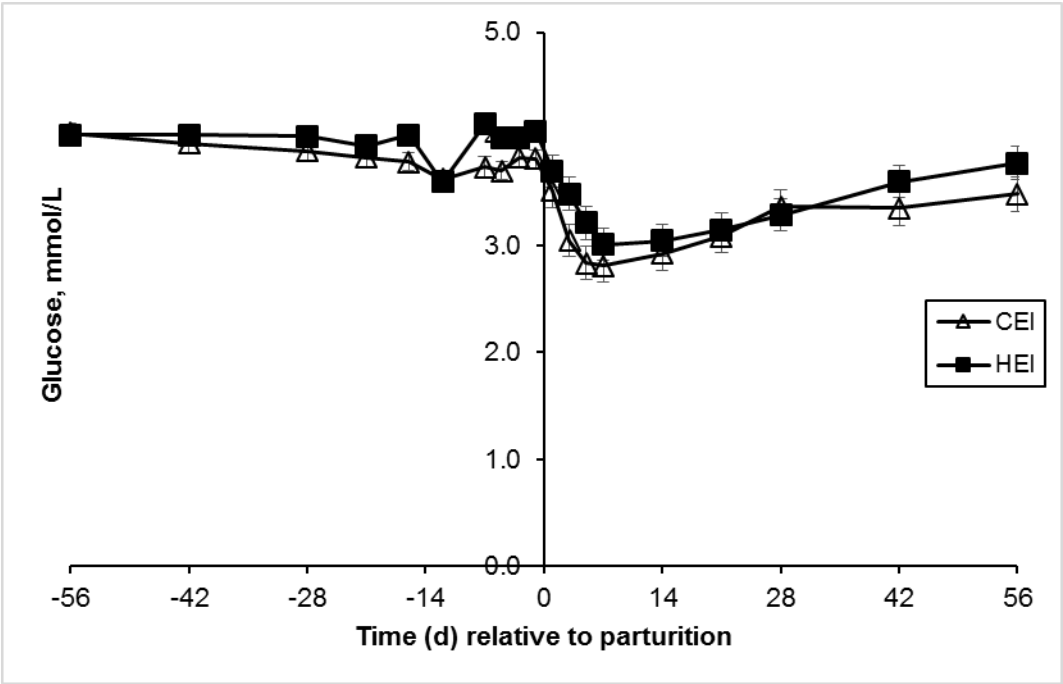
<sup>4</sup> Day = number of days to expect parturition; <sup>5</sup> n = 7 for NEFA statistics; \*P-values after natural logarithmic transformation; \*\* P-values from Friedman's non-parametric testing.

Salin, Figure 1 and 2

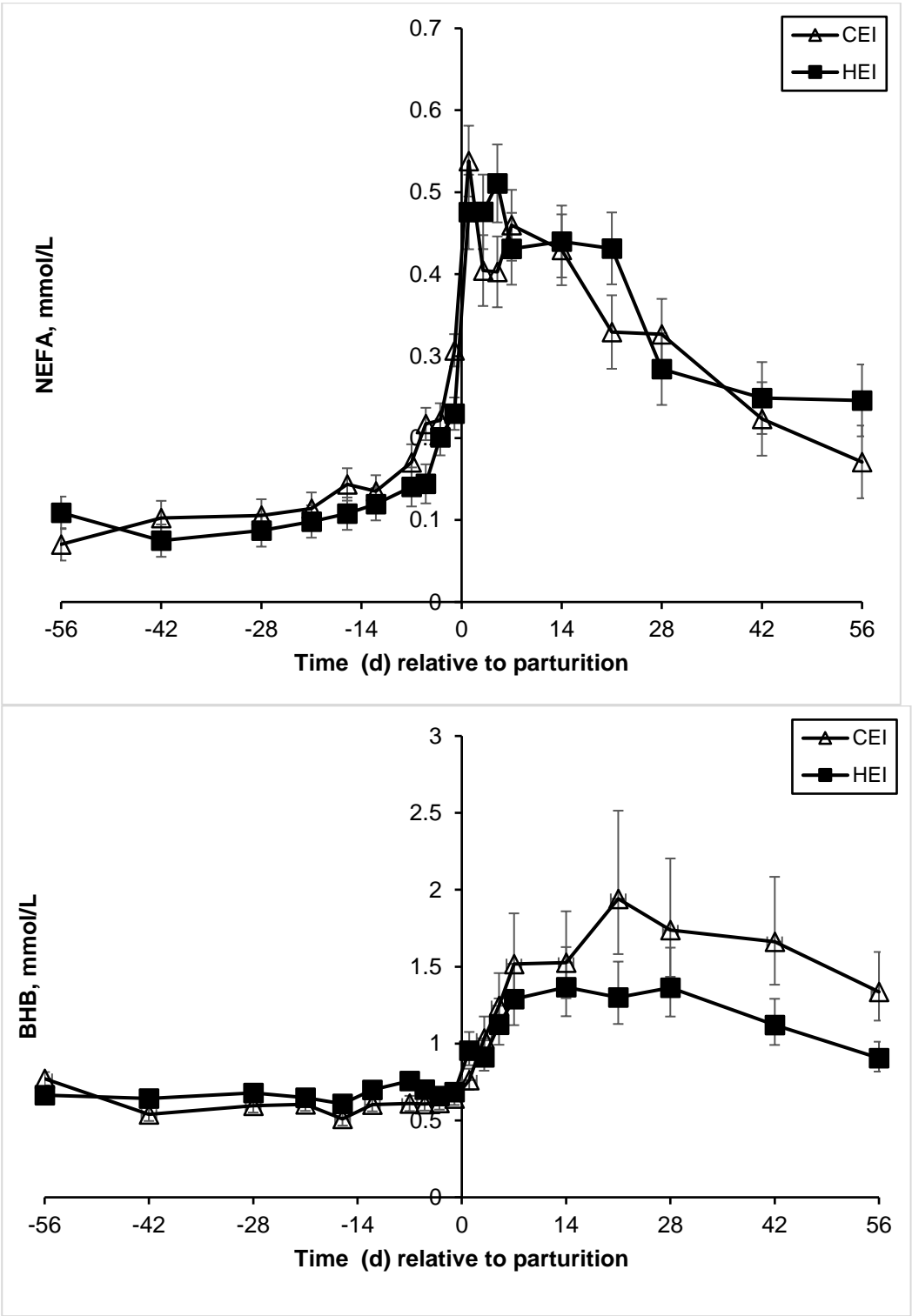




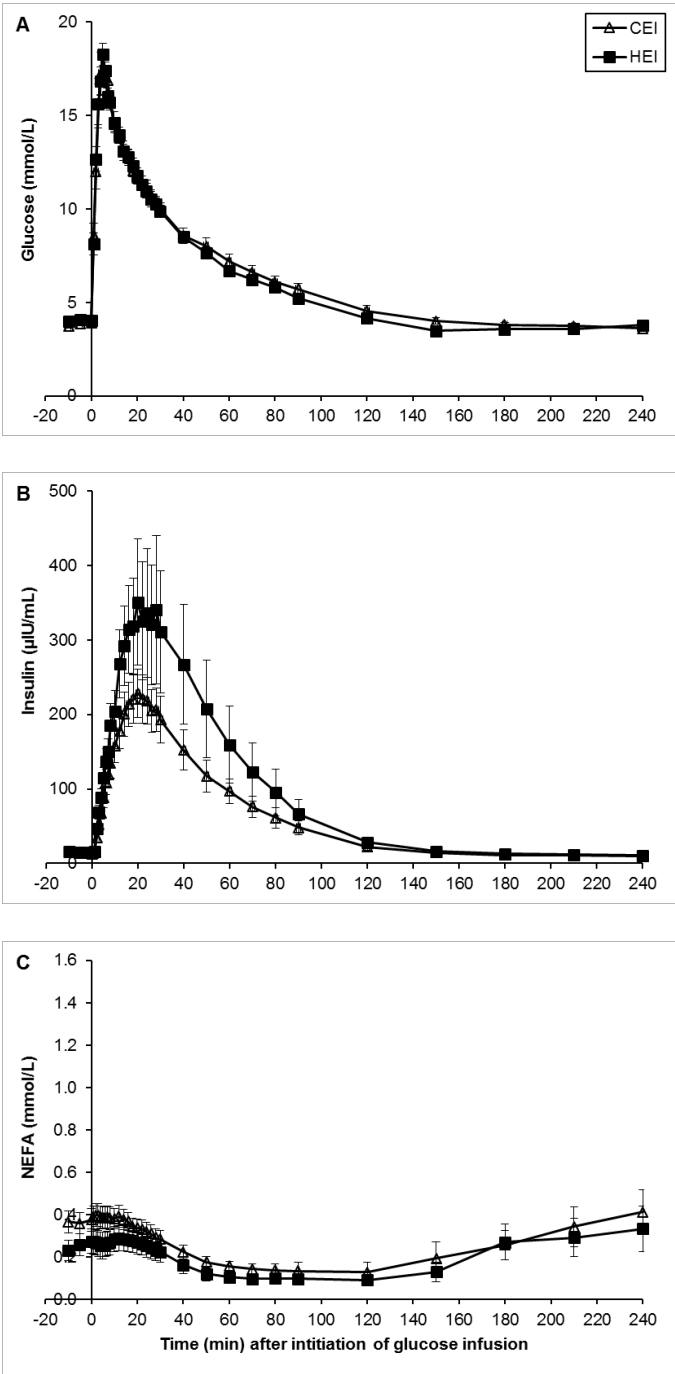
Salin, Figure 3 and 4



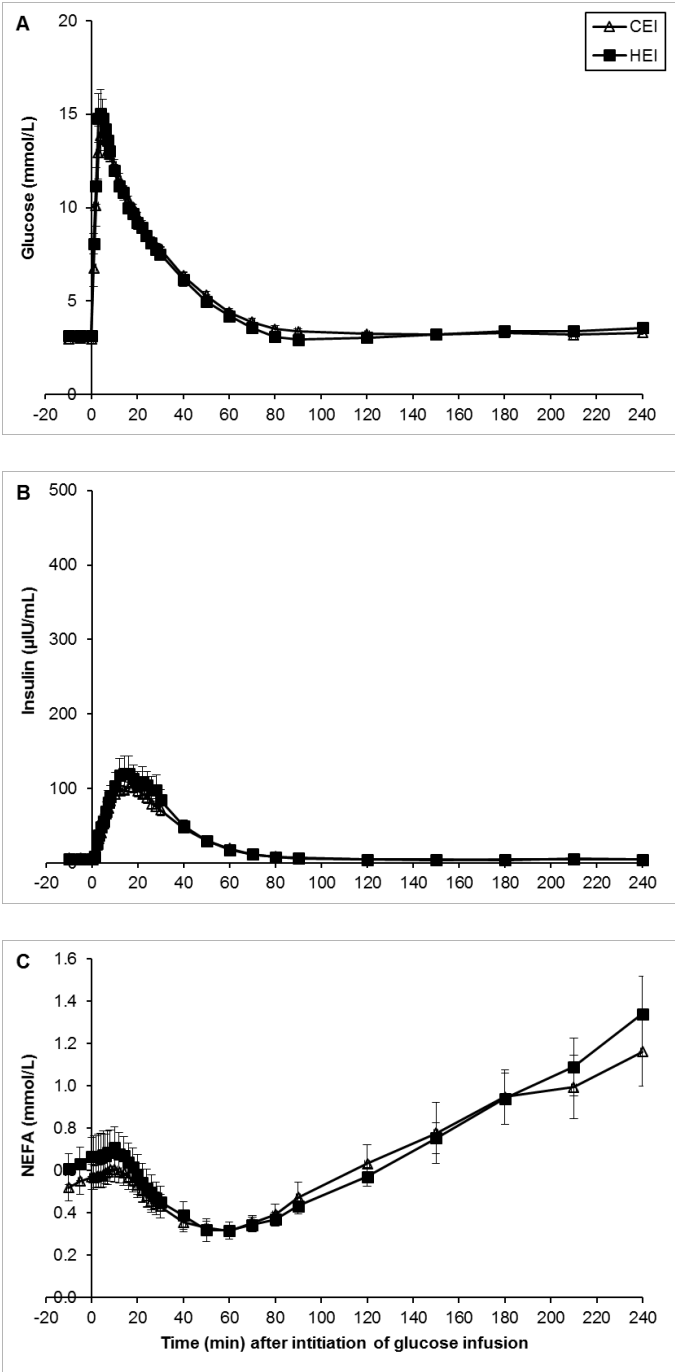
Salin, Figure 5 and 6



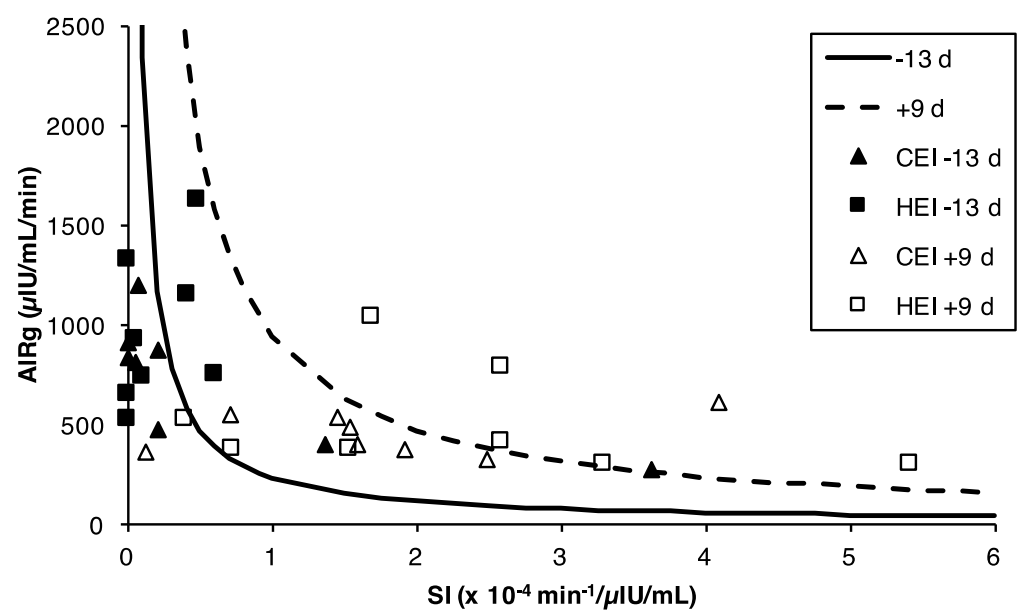
Salin, Figure 7A, 7B, 7C



Salin, Figure 8A, 8B, 8C



Salin, Figure 9



## Salin, Figure Captions

**Figure 1.** Dry matter intake of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM  $\pm$  SE of repeated measures analysis (n = 16). Pooled SE pre 0.50 kg/d, postpartum (pp) 0.57 kg/d. Effects of diet pre (P = 0.002), diet x time pre (P = 0.64), diet pp (P = 0.52), diet x time pp (P = 0.57).

**Figure 2.** Metabolizable energy balance of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM  $\pm$  SE of repeated measures analysis (n = 16). Pooled SE pre 4.8 MJ/d, postpartum (pp) 8.7 MJ/d. Effects of diet pre (P = 0.004), diet x time pre (P = 0.51), diet pp (P = 0.46), diet x time pp (P = 0.25).

**Figure 3.** Plasma glucose concentration of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM  $\pm$  SE of repeated measures analysis (n = 16). Effects of diet pre (P = 0.049), diet x time pre (P = 0.06), diet pp (P = 0.09), diet x time pp (P = 0.45).

**Figure 4.** Plasma insulin concentration of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are back-transformed LSM  $\pm$  SE from repeated measures analysis of log-transformed data (n = 16). Effects of diet pre (P = 0.07), diet x time pre (P = 0.08), diet pp (P = 0.52), diet x time pp (P = 0.37).

**Figure 5.** Plasma NEFA concentration of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are back-transformed LSM  $\pm$  SE from repeated measures analysis of log-transformed data (n = 16). Effects of diet pre (P = 0.26), diet x time pre (P = 0.07), diet pp (P = 0.62), diet x time pp (P = 0.28).

**Figure 6.** Plasma BHB concentration of cows fed two levels of energy in the dry period, CEI ( $\Delta$ ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI ( $\blacksquare$ ) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Prepartal values are LSM  $\pm$  SE of repeated measures analysis. Postpartal values are back-transformed LSM  $\pm$  SE from repeated measures analysis of reciprocally transformed data (n = 16). Effects of diet pre (P = 0.006), diet x time pre (P = 0.27), diet pp (P = 0.32), diet x time pp (P = 0.01).

**Figure 7.** Treatment effects on plasma (A) glucose, (B) insulin, and (C) non-esterified fatty acids (NEFA) concentration during intravenous glucose tolerance tests (0.25 g of glucose i.v./kg of BW) performed 13  $\pm$  5 d before parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI ( $\Delta$ )] and in cows fed 141% of the ME requirements of grass silage [HEI ( $\blacksquare$ )], during wk 8 to 1 prepartum. Error bars represent SEM. Least squares means of area under the curve for glucose, insulin, and NEFA in CEI and HEI were 525 and 413  $\pm$  47.9 mmol/L x 240 min, 10064 and 17762  $\pm$  3520  $\mu$ IU/mL x 240 min, and -20.0 and

$-13.4 \pm 4.3$  mmol/L x 240 min ( $n = 16$ ), respectively. Concentration at time point -5 min represents the average concentration at 10 and 5 min before IVGTT.

**Figure 8.** Treatment effects on plasma (A) glucose, (B) insulin, and (C) non-esterified fatty acids (NEFA) concentration during i.v. glucose tolerance tests (IVGTT; 0.25 g of glucose i.v./kg of BW) performed  $9 \pm 1$  d after parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI ( $\Delta$ )] and in cows fed 141% of the ME requirements of grass silage [ HEI ( $\blacksquare$ )], during wk 8 to 1 prepartum. Error bars represent SEM. Least squares means of area under the curve for glucose, insulin, and NEFA in CEI and HEI were 374 and  $322 \pm 30.1$  mmol/L x 240 min, 3063 and  $3884 \pm 561$   $\mu$ IU/mL x 240, and 35.0 and  $18.7 \pm 17.3$  mmol/L x 240 min, ( $n = 16$ ), respectively. Concentration at time point -5 min represents the average concentration at 10 and 5 min before IVGTT.

**Figure 9.** The hyperbolic relationship between the minimal model–derived indices of acute insulin secretion (AIRg) and insulin sensitivity index (SI) denoted as disposition index (DI) during the intravenous glucose tolerance tests (0.25 g of glucose i.v./kg of BW) performed  $13 \pm 5$  d before and  $9 \pm 1$  d after parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI ( $\Delta$ )] and in cows fed 141% of the ME requirements of grass silage [ HEI ( $\blacksquare$ )], during wk 8 to 1 prepartum. The hyperbolas were generated from extrapolated values of insulin secretion (AIRg) based on the average of observed values of DI for  $-13$  d ( $n = 16$ ), and  $+9$  d ( $n = 16$ ), and varying SI in the range from 0.01 to 6 (Stefanovski et al., 2011). All observations of SI and AIRg and the corresponding hyperbolas before and after parturition are represented by the symbols defined in the figure.